domain, and the PIK domain, but no clear function has been ascribed to this region. The paired arrangement of a series of helices connected into a right-handed super helix is reminiscent of the PR65/A regulatory subunit of protein phosphatase 2A (PP2A) (20). PR65/A is a member of a diverse group of proteins that contain between three and 25 tandem repeats of a short sequence that has been termed the HEAT motif. The HEAT motif consists of paired helices A and B arranged so that the A and B helices within a pair are anti-parallel and the A and B helices from one motif are parallel to the A and B helices of the next motif in the sequence. Although no HEAT sequence motif is apparent in the helical domain of PI3K, its structure is quite similar to that of PR65/A in terms of the arrangement of helices, the length of the A/B units, and the angle between the A/B pairs.

The function of HEAT repeats is to form protein/protein interactions. In the case of importin-β, the interactions that the protein makes with the small GTPase Ran involve primarily the surfaces of the B helices (21). For PR65/A, mutagenesis has implicated the loops connecting the A/B pairs as the region responsible for interaction with PP2A (20). In PI3Kγ, the helical domain is central to the inter-domain packing. The surface formed by the A helices interacts with the catalytic domain. The loops connecting A and B helices within a pair pack against the C2 domain while the loops between helical pairs pack against the RBD (Fig. 7). Much of the "B" surface is solvent-exposed and may interact with other proteins known to bind PI3Kγ such as the p101 adaptor or Gβγ subunits.

The helical domain is common to both PI3K and PI4K families and serves as a spine on which the other domains are fastened. One of the proteins in which the HEAT sequence motif was first noted is the target of rapamycin, TOR, a yeast

homologue of human FRAP (reviewed in (22)). FRAP has a C-terminal domain with clear sequence homology to the catalytic domain of PI3Ks. The secondary structure prediction for the remainder of FRAP suggests that most of FRAP, apart from the catalytic domain, may consist of helical repeats folded into a right-handed superhelix as observed in the helical domain of PI3Kγ.

This first view of the structure of a PI3K provides a framework within which mutagenesis and detailed kinetic studies can be carried out to establish the enzymatic mechanism and the mode of activation by Ras and heterotrimeric G protein subunits.

Methods

Protein expression, purification and crystallisation. The cell-free extract of baculovirus-infected Sf9 cells expressing the His-tagged catalytic subunit of porcine PI3Kγ (residues 1-143 deleted) was used for protein purification using Talon resin, followed by thrombin cleavage, anion and cation exchange, and gel filtration chromatography. Crystals were grown by mixing 1μl of PI3K (at 3.5 to 4.0 mg/ml, in a buffer containing 20 mM Tris-HCl pH7.2, 1% v/v ethylene glycol, 1% w/v betaine, 0.02% w/v CHAPS and 5 mM DTT) with 1μl of a reservoir solution containing 150-200 mM Li_2SO_4, 100 mM Tris-HCl pH 7.25 and 14-15% PEG 4000.

Data collection and structure determination. Crystals have C2 symmetry with unit-cell dimensions of a=143.3 Å, b=67.6 Å, c=107.0 Å, β=95.9°, and contain one protein molecule in the asymmetric unit. Diffraction data were collected at ESRF beamlines ID2 and ID14-4. Data were collected at 100K after freezing crystals in a cryoprotectant consisting of 150-200 mM Li_2SO_4, 100 mM Tris-HCl pH 7.25, 12% glycerol and 20% PEG 4000. Data were processed using MOSFLM (23) and CCP4

programs (24). The structure was determined by multiple isomorphous replacement (MIR) methods. Heavy-atom positions were located using Solve (25) and refined with Sharp (26) (Table 1). A model was built into the electron density maps using the program O (27) and refined using CNS (28). The average B-factor for all atoms is 60 $Å^2$. The structure has no residues in disallowed regions of the Ramachandran plot.

The highest resolution data obtained were for the complex containing ATP and lutetium. Refinement of this complex resulted in a model with a free R-factor of 0.30 to a resolution of 2.2 Å. This complex has 854 residues visible in the electron density map. Comparison of crystals with and without ATP showed only minor differences in side-chain conformations in the active site residues. PI3Ks require a Mg^{2+} or Mn^{2+} cofactor for enzymatic activity. Complexes with Lu^{3+}, Mg^{2+} or Mn^{2+} show that each of the metals binds at the same two sites.

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